MONITORING BIOLOGICAL DAMAGE ON PAPER-BASED DOCUMENTS IN THE HISTORICAL ARCHIVE OF THE PALERMO ASTRONOMICAL OBSERVATORY

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Keywords: paper biodeterioration; non-invasive sampling; molecular investigation

1. Introduction

As part of the framework in the fourth year of the Book Conservation course at the University of Palermo, the students' practical activities were hosted by the Palermo Astronomical Observatory, which possesses a rich historical archive and library. The Observatory of Palermo is still located in its original 18th century premises in the medieval tower of the royal palace, consequently, the presence of instruments, books and documents that have been there since its foundation, today makes it an extremely interesting place to train the next generation of conservators [1].

The careful conservation of the building and the set-up of a proper museum and library for this long-established institution has helped solve some major problems that affected the collections in the past.

When the books, old instruments and documents lost their scientific value, they were not always kept in optimal conditions, a factor that reflects their present state. In a constant effort to preserve their wide ranging heritage, the Observatory welcomed the presence of three students in book conservation together with their tutor. The Observatory management was also able to supplement some funds to purchase special materials needed to carry out the project. The aim was to give the students experience in activities related to what is called collection care. This involves conservation assessment and stabilization of the damage on a large number of items in order to extend the life of a collection before it needs extensive or major conservation treatment.

This work was carried out on several documents on paper and parchment, on printed and manuscript books and on a number of glass plate negatives. Each item's condition was assessed using a specifically designed survey form, photographed and evaluated for treatment. When treatment was possible *in situ*, it was carried out in the form of dry cleaning and small repairs. Among the treated documents, several showed previous mould damage.

Library and archive material is prone to different kinds of damage which is mainly physical due to the normal use of the items, chemical, physical and biological when

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environmental conditions are not appropriate or as a result of some accident. Biological damage is one of the higher risks in paper-based collections because attention to regular cleaning and monitoring is often absent or neglected. When biological attacks occur they affect the substrates and graphic media, creating stains and discolouration. If the damage is not detected early enough and promptly treated, it can lead to the total destruction of the object [2-4]. Cellulose and collagen-based materials, in certain conditions, are mostly susceptible to fungal and bacterial deterioration, due to the action of specific enzymes (cellulose, proteases, ligninases) and organic acids [5, 6].

Fungal contamination is also considered a concern because the dispersion of spores can contaminate the environment and may produce allergies and other illnesses in humans [6, 8]. Depending on the environment, different fungal genera can be found, considered to be producers of dangerous mycotoxins that can affect eyes and ears [4, 6]. The environmental microorganisms that form the microbial load of indoor air at repositories (archives, libraries) storing cultural heritage, can affect human health in the form of allergies and skin affections [9, 10]. Biodeterioration and health risks justify the need to perform periodical microbiological samplings to estimate the prevalence of microbial contamination [7, 11]. Knowledge and understanding of the materials from which documentary heritage is made and the identification of damage are fundamental to carry out any proper conservation treatment [5].

The aim of this study was therefore, to identify any cultivable microorganisms isolated from documents in the Archive of Astronomic Observatory in Palermo, in order to estimate levels of microbial contamination on the surface of several stored items. This research combines information from molecular biology with microbial morphology aimed at identifying infecting fungi and bacteria from heritage documents. Four archive items, previously colonised by moulds and restored were selected for non-invasive samplings by sterile swabs and fragments of nylon membranes in order to detect the main microbial re-colonisation [12].

This work focuses on the biological contamination of paper collections and documents and suggests a non-invasive and rapid system of sampling and diagnosis which can be adopted for the monitoring of different environments and cultural objects.

2. Materials and methods

The sampling was performed in the Astronomical Observatory Historical museum (Palermo). Environmental temperature (T) and relative humidity (RH) were measured during sampling, using a digital thermo-hygrometer.

Microbiological sampling was carried out on the surface of 4 historical documents:

i. ***AMP*** - *Institutionum Philosophicarum pars tertia utramque Phisicam complectens*. Bound paper manuscript, 1767 (shelf mark: Cart. 78 Fasc. 1);
ii. ***IMP*** - *[Boucher, Antoine-Gaspard: d'Argis] Manuale dei periti in materie civili* [*Palermo: dalla tipografia degli eredi Abbate*, 1828]. Printed book on paper (Inv. 10960/BAOA);
iii. ***ALE*** - *Catalogo de' Libri esistenti nella Biblioteca del Reale Osservatorio di Napoli; Inventario degli Stromenti Mobili e di ogni altra cosa esistente nel Reale Osservatorio di Napoli*. Manuscript quire on paper [1820] (shelf mark: Cart. 77 Fasc. 8);
iv. ***ALP*** - *Catalogo dei Libri dal P. Piazzi lasciati all'Osservatorio*. Manuscript quire on paper [1817] (Cart. 60 Fasc. 2).

Table 1 lists the investigated documents and reports the main alterations of the analysed documents (Figure 1).

Table 1. Biodeterioration evidence on analysed documents.

Sample	Type of document	Type of alteration
***AMP**, Institutionum Philosophicarum pars tertia utramque Phisicam complectens, 1767*	Bound paper manuscript	blackish, pinkish spots large grayish stain due to humidity loss of material
***IMP**, Manuale dei periti in materie civili, 1828*	Bound printed book on paper	black staining foxing spots loss of material
***ALE**, Catalogo de' Libri esistenti nella Biblioteca del Reale Osservatorio di Napoli, 1820*	Manuscript on paper	pigmented areas reddish and greenish spots and staining loss of material
***ALP**, Catalogo dei Libri dal P. Piazzi lasciati all'Osservatorio, 1817*	Manuscript on paper	large violet stain black pigmented area loss of material

Sampling of microbial (fungal and bacterial) structures from damaged archival documents was carried out using non-invasive methods, and in particular by: *i*) sterile nylon membrane fragments (10 cm2) (*Hybond, Amersham* H+) gently pressed (for 30 seconds) over the investigated area (Figure 2 A); *ii)* sterile swabs by wiping across spots showing visible damage and staining (Figure 2 B,C), and then used in culturing techniques to obtain *in vitro* growth of the microorganisms.

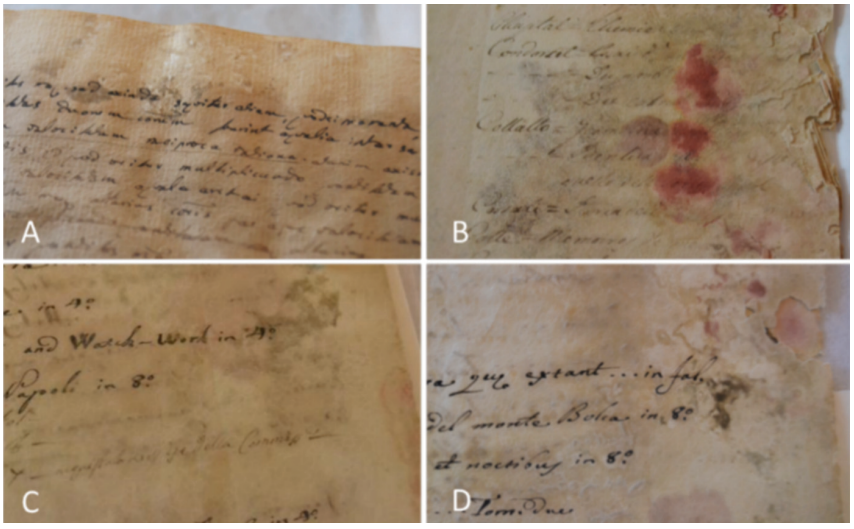

Figure 1. Paper alterations. (A) AMP sample, dark spots and damp patches; (B) ALE sample, reddish spots, and loss of material; (C-D) ALP sample, violet and greenish stains, and loss of material.

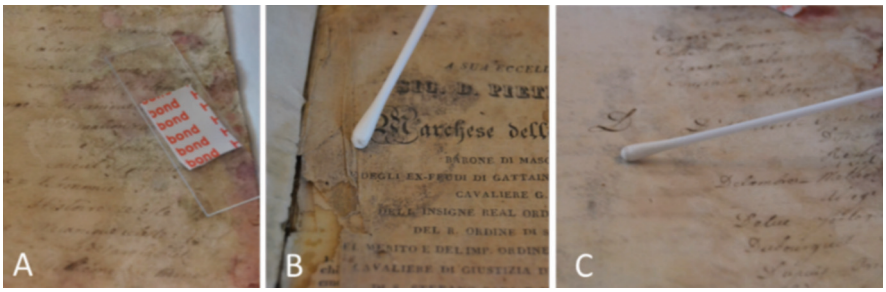

Figure 2. Non-invasive sampling on documents. (A) Using nylon membrane and (B-C) sterile swabs, in ALP and IMP samples, corresponding to pigmented areas and fragile portions of the paper.

For each document, two samplings using nylon membranes and one sampling using sterile swabs were performed in those areas mainly affected by pigmentation, discoloration, foxing phenomena and loss of material.

After sampling, the nylon membrane fragments were immediately transferred onto Petri-dishes containing nutritive solid media in order to isolate bacteria (Nutrient Agar, *Difco*) and fungi (Sabouraud, *Difco*). Microbial particles collected by sterile swabs were firstly suspended in culture liquid media (Nutrient Broth, *Difco*) and then seeded onto appropriate solid nutritive media. All Petri dishes were incubated at 30°C for 48-72 hours.

2.1 Microbial identification

The identification of microbial taxa was carried out using an integrated approach based on *in vitro* culture, microscopy analysis and molecular investigation. Particularly, filamentous fungi structures and reproductive propagules were observed through optical microscopy (Leica, 40X) after Lugol's solution staining [12].

Phylogenetic identification was performed by molecular investigation based on microbial genomic DNA analysis. DNAs were directly extracted from bacterial and fungal colonies using a commercial kit (Genomic DNA Purification kit, *Fermentas*) and utilized as templates in *in vitro* amplification of specific genomic target sequences by Polymerase Chain Reaction (PCR). Particularly, the target sequences correspond to the Internal Transcribed Spacer 18-26S for fungi and 16-23S for bacteria. PCR products were analyzed on agarose gel (2%) in TAE solution (TRIS-HCl/acetate/EDTA).

PCR products were sequenced by Eurofins MWG Operon sequencing-service and the sequences analysed by BLAST platform [12, 13].

3. Results

Several microorganisms (bacteria and fungi) were isolated using culture-dependent techniques and characterized by molecular investigations, as reported in Table 2.

The characterization of isolated fungi was carried out through microscopic analysis (Figure 4), showing that *Penicillium* was the predominant genus in all documents, while *Aspergillus niger* was detected only in the AMP document.

Bacterial colonies belonging to *Bacillus* genera were isolated from all documents, distinguishing AMP (*Bacillus* spp.), IMP and ALE (*B. simplex, B. flexus*). *Micrococcus* spp. was revealed in IMP, and *Micrococcus luteus* in ALE and ALP. *Arthobacter* spp. was isolated only from the ALP sample.

Table 2. Isolated and characterized microorganisms.

Microorganisms	*AMP*	*IMP*	*ALE*	*ALP*
Fungi				
Aspergillus niger	+	-	-	-
Penicillium spp.	+	+	+	+
Bacteria				
Arthobacter spp.	-	-	-	+
Bacillus simplex	-	-	+	-
Bacillus flexus	-	-	+	-
Bacillus spp.	+	+	-	-
Micrococcus spp.	-	+	-	-
Micrococcus luteus	-	-	+	+

Presence (+); Absence (-)

Fungal taxa revealed in this study, belonging to Deuteromycetes, are environmental and widespread fungi, capable of colonizing several organic substrates (such as cellulosic materials) as a source of nourishment. Their presence in indoor environments, such as archives or libraries, could induce allergies and skin infections [14]. Bacteria isolated in this study are often found on organic materials producing stains of different shape and color and are generally associated with chromatic alterations.

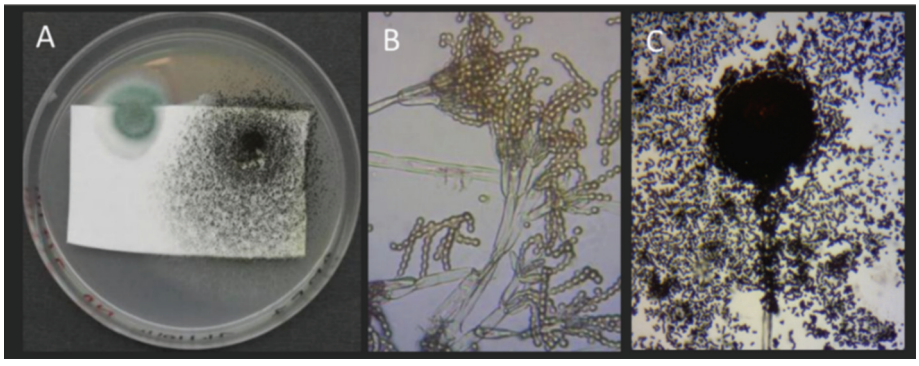

Figure 3. AMP samples, fungi colonies grown on nylon membranes in Sabouraud medium (A); Light microscopic observations (40X) following Lugol staining of (B) Penicillium spp. and (C) Aspergillus niger.

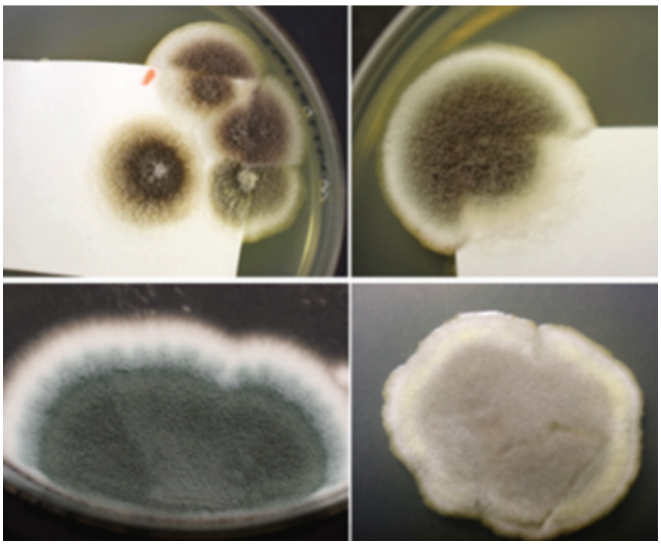

Figure 4. Macro-photos of Penicillium spp. colonies growing on nylon membranes after samplings from ALP and Petri dish growth.

4. Conservation treatment

Since the microorganisms responsible for the biological damage were no longer active and the original material was not wet, it was possible to carefully dry clean by brush every single page. The procedure was carried out in an open area to prevent spread of the spores within the museum environment. This allowed the deposits and mold remains to be removed [15]. In the areas that were more severely affected by the infection the support had suffered from partial weakening and thus required some consolidation. Since simple re-sizing was not enough to give the necessary flexibility and support to the affected area, it was decided to line the fragile area with Japanese tissue, only on one side. The use of a water-based adhesive was avoided due to the weakness of the paper and also to avoid introducing unnecessary moisture on an area already affected by microbiological attack. Klucel G (hydroxypropyl cellulose), diluted in ethanol (3% w/v) was then selected and applied by brush over the Japanese tissue.

Many of the items treated during the training period were provided with a custom-made archival cardboard folder or box. The use of protective enclosures reduces the impact of the environment on the items. A box or a folder will stop the direct effect of light and dust; it has been proved that enclosures also have a mitigating effect in the event of fires and floods. All the enclosures were made of specifically produced folding box board, with enhanced wear and tear strength so as to endure the mechanical stress of the opening and closing of the container and to resist abrasion from shelving and handling. The cardboard is made of pure cellulose with alkaline buffer to be chemically safe for the boxed contents and at the same time, being an organic porous material, it absorbs and releases moisture before it can come in contact with the documents, playing a buffering action for environmental fluctuations (Figure 5).

Figure 5. Protective boxes for correct storage of archival documents.

5. Conclusions

In the present study, a dry cleaning procedure and consolidation of some documents stored in Palermo Astronomical Observatory's historical archive and library were carried out. Moreover, optical, culture-based and molecular analyses were carried out to determine the extent of the microbial deterioration of the paper-based objects. Discoloration and coloured spots, mainly reddish and blackish, alterations probably due to the microbial activity (past or present) were observed on investigated paper-based objects. Dark fungal isolates of *Aspergillus niger* were identified after observation of the black-spotted sampling areas (in particular the AMP sample). In all samples, *Penicillium* spp. was the main species isolated.

In relation to bacteria, *Bacillus* spp. was the predominant species able to excrete hydrolytic enzymes and has already been isolated from archives affected by foxing and paper [16]. *Arthobacter* spp. and *Micrococcus* spp. contamination was found mainly in ALE and in ALP samples; in paper, their metabolic activity can induce discoloration and staining. These microbial taxa can contribute to structural degradation, acting both mechanically (fungal hyphae growth) or chemically (microbial enzymes or acid extraction).

However, the total microbial loads were considered of no risk for the integrity of the analyzed documents. Most of the structural alterations and stains are the result of past colonization by some microbial deteriogens that are no longer metabolically active.

To prevent further occurrence of biological damage, regular cleaning, periodical assessment and environmental monitoring is recommended. In addition, the buffering action of the protective enclosures will prevent the effect of possible fluctuations in environmental parameters and protect from dust and spores, thereby, further reducing risks.

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Biographical notes

Marco Di Bella, freelance book conservator, graduated at the European Course for Conservators-Restorers of Book Materials in Spoleto (Italy) in 2001; he has worked in conservation, assessment and training projects for the Camberwell College of Arts (UK), UNESCO, Yemeni Social Fund for development, ISCR, ICPAL, National Archive of Tripoli (Libya), University of Palermo, the Hiob Ludolf Center for Ethiopian Studies (Hamburg University), the American University of Beirut. He has worked for private book conservation studios in Italy and lectured in international conferences, researched and published on archaeology of early Islamic bookbinding and book conservation. Since 2015, he is senior manuscript conservator at Trinity College, Dublin.

Donata Randazzo has a degree in Biology and graduated as a "Librarian" in England, she is librarian of Palermo Astronomical Observatory, where she takes care especially of the Old and the Historical Fund. She collaborated in the compilation of the biographical repertoire of Italian astronomers and inventory of the Palermo Observatory Historical Archive.

Enza Di Carlo has a Master's Degree in "Biological Science" from the University of Palermo and specialized in "Microbiology and Virology" at the School of Medicine at the same University. She is a Research Fellow at the Laboratory of Biology and Biotechnologies for Cultural Heritage of the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) at the University of Palermo, where she has carried out microbiological monitoring of Cultural Heritage environments.

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Summary

Libraries and archives house a wide variety of documents made of materials of vegetal and animal origin: unbound papers and books, manuscripts and printed books, photographs (negative and positive), prints, maps, available to the public for reading needs, study and information. These materials are often subject to fluctuations in environmental and micro-environmental parameters. For this reason, it is essential to implement constant monitoring and control of environmental conditions and potential deteriogens in order to slow down deterioration processes.

The monitoring of the microbial degradation of paper documents in the Historical Archives of Palermo Astronomical Observatory has revealed microorganisms (bacteria and fungi) that may be considered responsible for damaging the items examined, thus enabling an evaluation of the real risks and the proper methodologies to use to avoid future recolonization.

Riassunto

Gli archivi e le biblioteche custodiscono diverse tipologie di documenti composti da materiali di origine vegetale e animale: carte sciolte e volumi, sia manoscritti che a stampa, fotografie (negativi e positivi), mappe, stampe, a disposizione del pubblico per esigenze di lettura, studio e informazione. Questa varietà di materiali sono spesso sottoposti a parametri ambientali e micro-ambientali non costanti. Per questo motivo è fondamentale operare sia un controllo costante delle condizioni ambientali sia un campionamento microbiologico sulla superficie, al fine di rallentare i processi di deterioramento.

Il monitoraggio del degrado microbiologico di documenti presenti nell'Archivio Storico dell'Osservatorio Astronomico di Palermo ha permesso di rilevare diversi microrganismi (batteri e funghi) che possono essere considerati responsabili del degrado dei documenti analizzati, valutando il reale rischio e le metodologie più idonee per impedire la ricolonizzazione futura.